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Despite admixing two closely related *Carex* species differ in their regional morphological differentiation

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Abstract

Rarer species are expected to show stronger geographic differentiation than more common species. However, if rare species hybridize with common species, differentiation may be quite similar between the two due to genetic admixing via backcrossing. We studied morphological differentiation of plants of 21 natural populations of the more common *Carex flava,* 16 of the less common *C. viridula* and 6 of their hybrids from 27 sites in three climatically different regions, Estonia, Lowland Switzerland and Highland Switzerland. Univariate ANOVA and multivariate Principal Component Analysis of 14 morphological characters, describing both vegetative and reproductive characters, allowed to clearly distinguish *C. flava* from *C. viridula*. *Carex viridula* populations showed stronger regional variation than *C. flava*. Hybrids had both intermediate and transgressive characters in Switzerland and Estonia. On average, hybrids from Lowland Switzerland were more similar to Swiss *C. flava* than to *C. viridula*, while hybrids from Estonia were morphologically intermediate between plants of Estonian populations of the parental species. The results suggest that within-region genetic admixing between species has limited potential to lead to region-specific similarity between species, at least in our model system of the *C. flava* complex. We conclude that *C. flava* and *C. viridula* are clearly distinct species and that, despite hybridization, geographic differentiation is more pronounced in the less common *C. viridula* than in *C. flava*.

Keywords: admixing, morphology, *Carex flava* complex, hybridization, geographic differentiation

Introduction

Widespread plant species often display remarkable morphological variation across their area of distribution, which often spans considerable environmental gradients. Among possible causes are phenotypic plasticity or variation due to local adaptation (West-Eberhard 2003). Accordingly, plant phenotypic variation and performance have been found to change with latitude (Santamaría et al. 2003; Hall et al. 2007) and altitude (Clausen et al. 1948; Jonas and Geber 1999). Weaker geographic differentiation is expected for more common species with continguous distribution area and higher levels of gene flow than for less common species with scattered distribution and more isolated populations (Frankham et al. 2002). Patterns of phenotypic variation in higher plants are also influenced by hybridization and introgression between taxa (Anderson 1949; Arnold 2006). Hybridization and introgression can lead to genetic admixing (Lexer et al. 2010; Rius and Darling 2014; Hahn and Rieseberg 2016) between closely related and co-occurring species, reducing phenotypic differences.

At the same time, novel genetic combinations may emerge that present an important potential for evolution and speciation in plants (Stebbins 1959; Arnold 1994; Rieseberg 1997; Hegarty and Hiscock 2005). Hybridization between species has been widely studied, including examples of hybridizing irises (Arnold 1992, 1994), sunflowers, ragworts (Lowe and Abbott 2003), or *Helichrysum* species (Galbany-Casals et al. 2012). However, most studies concerned only geographically limited regions. It is not clear whether genetic admixing may lead to greater similarity between geographically close than between distant populations of hybridizing species. This would be especially interesting for pairs of common and rare species co-occurring over large stretches of their ranges, as the expectation of higher regional differentiation in the rare species might be counter-balanced by introgression from the more common species. Possibly, if genetic admixing is strong between close populations of the two species, within-species variation between regions might even exceed between-species variation within regions. In addition to these hypotheses, as hybrids are mosaics of parental, intermediate and transgressive characters, this large variation may lead to differential selection in different regions (Rieseberg et al. 1993; Rieseberg et al. 1999; Rosenthal et al. 2002).

Carex flava L. and *C. viridula* Michx. var. *viridula* are two taxa in the *C. flava* complex (Cyperaceae) with distribution in the temperate and subarctic Northern hemisphere and also in North Africa (Davies 1953b; Hultén and Fries 1986; Crins and Ball 1989b). In Europe, both taxa occur from Scandinavia and the Baltic in the North to the Mediterranean in the South (Koopman 2011). In Estonia and in Switzerland *C. flava* is more common and abundant than *C. viridula* (Schmid 1981; Kukk and Kull 2005). *Carex viridula* has a more narrow ecological

distribution and constitutes smaller populations of more scattered geographic distribution (Davies 1955; Schmid 1984b, 1984a; Kuchel and Bruederle 2000). Davies (1953c) considers it to be a relict of wider distribution in the past, which disappeared from many localities due to habitat loss and alteration through peat mining, drainage of wetlands, regulation of water level and eutrophication of lake shores, where *C. viridula* prefers to grow (Pykälä and Toivonen 1994; Kuchel and Bruederle 2000).

While both are considered distinct taxa, they are highly polymorphic and differences are subtle with many individuals being morphologically intermediate (Egorova 1999). The morphology of the *C. flav*a group species has therefore been addressed, e.g. in the British Isles (Davies 1953b; Blackstock and Ashton 2010), in Bulgaria and former Czechoslovakia (Stoeva and Stepankova 1990), in Finland (Pykälä and Toivonen 1994), in Fennoscandia (Hedrén 2002), in Spain (Jiménez-Mejías et al. 2012, 2014), in Poland (Więcław 2014), in Switzerland (Schmid 1980, 1983) and in North America (Crins and Ball 1989b, 1989a). However, the amount of regional morphological variation between climatically different geographic regions, and whether it differs between taxa, is not clear. Accordingly, 2012 (2012), who consider *C. flava* and *C. viridula* as distinct species, recommended future research based on expanded geographic sampling.

Hybridization between *C. flava* and *C. viridula* is likely as their flowering periods overlap to a considerable extent, even though *C. viridula* flowers slightly later in the spring than *C. flava* does (Vonk 1979). Hybridization might also have played an important role in speciation in the section (Davies 1955) and has been linked to the instable chromosome numbers observed in *C. flava* populations from sites with co-occurring *C. viridula* in Switzerland (Schmid 1982). Indeed, backcrossings of the hybrid with either parent are leading to introgressive hybridization between taxa of the *C. flava* complex, i.e. to admixing, which results in morphologically similar forms to the parents (Schmid 1982, 1983). Accordingly, we found admixture of alleles between *C. flava* and *C. viridula* in a parallel study of microsatellite differentiation (Schmidt et al. in prep.). Hybridization in sedges may have recently increased quite dramatically as a consequence of anthropogenic habitat disturbance (Cayouette and Catling 1992). Hybridization between members of the *C. flava* complex was reported across their distribution ranges (Schmid 1982, 1983; Crins and Ball 1989b; Hedrén 1990; Egorova 1999) and was demonstrated to result in morphologically intermediate individuals (Stoeva and Stepankova 1990; Blackstock and Ashton 2010; Więcław 2014). Studying the differentiation between hybrids from different regions would show whether the hybrids evolve differently in different regions and thus add to local biodiversity.

To address morpho-geographic differentiation within and between the hybridizing species *C. flava* and *C. viridula* var. *viridula* we sampled plants in three climatically different regions, in Estonia, Lowland Switzerland and Highland Switzerland. We addressed the following questions: (1) is regional differentiation more pronounced in *C. viridula*, whose populations are more isolated and smaller in size? (2) Is between-region differentiation within species comparable in magnitude to within-region variation between species? (3) Are hybrids and their regional differentiation intermediate between parental taxa?

Material and methods

Study taxa and sampling

Species in the section *Ceratocystis* Dumort., to which the *C. flava* complex belongs, are relatively small sedges. *Carex flava* and *C. viridula* are polycarpic perennials, grow clonally via very short stolons and thus form tight tussocks of genetically identical vegetative and reproductive tillers (Schmid 1984a). Flowers are arranged on condensed spikes, which each usually contain flowers of only one sex. One staminate spike is normally terminal, underneath are 2–4 clumped pistillate spikes. Beneath the inflorescence is a long, spreading or down-turned bract. Female spikes consist of perigynia (or utricles), which contain single seeds in nuts. The utricles narrow abruptly to a distinct beak. Lower utricles are commonly down-turned when ripe. All taxa of the *C. flava* group are wind-pollinated, monoecious, and self-compatible (Vonk 1979). While the inflorescences are protogynous, partial autogamy via geitonogamy is rather common, so that about half of all seeds typically result from self-pollination (Schmid 1982). Both allogamy and autogamy lead to normal offspring (Schmid 1982). *Carex flava* occurs in bogs, fens, wet grasslands and ditches, and *C. viridula* additionally on shores of ponds and lakes with low vegetation cover due to flooding (Schmid 1980; Crins and Ball 1989b, 1989a; Pykälä and Toivonen 1994; Toom et al. 2016).

From 2012–2013, we sampled *C. flava* and *C. viridula* var. *viridula* sensu stricto (henceforth *C. viridula*), and their hybrids (*C.* x *ruedtii* Knecker = *C.* x *subviridula* Fernald) from 27 natural sites; 11 in Estonia up to 100 m a.s.l, five in Lowland Switzerland between 250 and 1000 m a.s.l and 11 in Highland Switzerland between 1000 and 2000 m (Table 1a-c). Estonia (57°–59° N) is situated in a transition zone between maritime and continental temperate climates with moderate temperatures during the 5–6 months of vegetation season and with predominant summer precipitation. Switzerland (45°–47° N) was divided into two parts based on elevational difference. In Lowland Switzerland below 1000 m a.s.l. the temperate climate is mild, warm and moist, the vegetation season is long and freezing temperatures occur only

during a short period in winter. The cold and moist environment of Highland Switzerland is harsh with a short vegetation season (Körner 1999).

We sampled the plants in various types of habitats, *C. flava* in populations in bogs, fens, wet grasslands and inundated ditches, and *C. viridula* additionally also on lake shores. As expected, populations of *C. flava* were larger than populations of *C. viridula* in all three regions. The southernmost population was in Caslano in Lowland Switzerland (45^0 57` N; canton Ticino) and the northernmost one in Anija in Estonia ($59^0$ 17` N; Harju county). In Estonia we found most of hybrids occuring also in disturbed sites (e.g. drainage, establishment of trails or artificial lakes), e.g. in Luunja, Estonia, on the shore of an artificial lake, or in Leie, Estonia, in an inundated ditch, in the Risti dried bog, Estonia, next to a highway and in Robenhuserriet, Lowland Switzerland, adjacent to a trail through a wet meadow. As hybrids, we considered sterile individuals with empty discolored utricles, growing at sites where both parent species were present. The hybrids were robust plants with sturdy leaves whose overall color was pale green not yellowish green like *C. flava* or like pure green *C. viridula* (L. Schmidt personal observation). With the use of molecular markers we found some individuals having admixed genotypes (Schmidt et al. in prep.).

Depending on the size of the population, we collected 5–30 mature individuals per population. Because taxa of the *C. flava* complex have very short rhizomes, samples obtained from discrete tussocks were assumed to represent different genetic individuals ("genets" in the terminology of Harper (1977). At 17 sites only one of the two species occurred, whereas at the other ten sites *C. flava* and C. *viridula* occurred together and at six of these their hybrid was also present. In Highland Switzerland *C. flava* and *C. viridula* co-occurred at three sites, where we did not detect apparent hybrids. Thus, we compare the two parental taxa between all three regions, but compare them to the hybrids only for Lowland Switzerland and Estonia.

All collected plants were dug out and immediately screened for 14 morphological characters. Then all populations were cultivated and maintained in two common gardens, one at the Universities of Zurich, Switzerland, and one in Tartu, Estonia. Morphological variation of populations in the experimental garden is not a subject of this paper, but will be a follow up study.

Phenotypic measurements

All morphologically investigated plants, i.e. all plants collected in the field in 2012/13, were measured at fruiting stage. We measured fourteen characters on reproductive tillers (Table 2), five vegetative (leaves and stem measurements) and nine floral ones (inflorescence-related measurements). We chose these morphological characters based on previous morphometric

studies (Davies 1953b; Schmid 1983; Crins and Ball 1989a; Pykälä and Toivonen 1994; Hedrén 2002). We measured macro-morphological characters on the fresh specimens with a standard ruler at an accuracy of 1 mm (e.g. length of stem, leaf and bract). The utricles were dried and photographed with an Olympus SP-500UZ digital camera mounted on an Olympus SZX7 binocular stereo microscope system before five well-developed utricles per plant were measured on the digital image with the Quick Photo Micro 2.2 software (Promicra). We deposited voucher specimens of all 43 populations in the herbarium of the Institute of Ecology and Earth Science of the University of Tartu (TU).

Data analysis

All analyses were carried out using software R, version 3.1.2 (R Core Team 2015). Variation in quantitative phenotypic characters was analyzed with three methods. First, we used mixed-effects ANOVA to examine the effects of the fixed factors "Taxa" and "Region" and their interaction on each trait, with "Population" as random factor. Only staminate-spike peduncle length (SSPL) needed to be log-transformed prior to the analysis to meet the ANOVA assumptions of homoscedasticity and normality. For the qualitative characters we did the same analysis, but based it on population means. The analysis was carried out separately for the two parental taxa in all three regions, and for the hybrids with parental taxa for the two regions, where hybrids occurred.

Secondly, the overall phenotypic variation among (1) 526 individuals of *C. flava* and *C. viridula* in three regions and among (2) 435 individuals of *C. flava*, *C. viridula* and their hybrids in two regions, across all 14 characters was analysed by Principal Components Analysis (PCA) based on the corresponding correlation matrices of the 14 characters for the separate (1) or combined (2) taxa. Using correlations implies character standardization to zero mean and one-unit standard deviation. We extracted principal components with eigenvalue > 1 and we recorded factor loadings with absolute value > 0.5.

Finally, principal component scores were calculated for each individual and these scores then analyzed with mixed-effects ANOVA as used above for the originally measured characters. To study how within- and between-population variation contributed to overall variation, we calculated variance components for both.

Results

Morphological differentiation between *C. flava* and *C. viridula*

Overall morphological differentiation between *C. flava* and *C. viridula* was significant for 10 of the 14 characters tested, and the five most discriminative ones all were reproductive

characters (Table 3a). Moreover, we found common regional differentiation in both species for three characters (LW, SSL, SC, see Table 2 for abbreviations) and a different regional differentiation between the two species for eight other characters (PH, LL, BL, FSW, UL, UW, UBL, UBC; significant interaction Taxa*Region in Table 3a). Among-population variance components varied between 11% and 40% of the sum of the within- and among- population residual variance (i.e. the variance after fitting taxa and region) for all characters, with higher values for vegetative characters (PH, LL) than for reproductive ones. In Lowland Switzerland and Estonia, the between-species differentiation was prominent in both vegetative and reproductive characters. In Highland Switzerland, however, the interspecific differentiation was evident only in the generally more constant female spike and utricle characters (Figs. 1, 2). This is because *C. flava* forms dwarf phenotypes at higher altitudes, sometimes referred to as *C. flava* var. *alpina* Kneucker (Schmid 1983), which are more similar to *C. viridula*.

The first two principal components explained 42% and 12% of the variation among the 14 morphological characters for all individuals of *C. flava* and *C. viridula* in Estonia and Lowland and Highland Switzerland combined (Fig. 3a). The centroids of *C. flava* from the three regions were closer together than those of *C. viridula*, indicating that despite hybridization and introgression the rarer species was differentiated more clearly between regions. However, observed interspecific differentiation was comparably weak in Highland Switzerland (Fig. 3a). Because their similarity is mainly expressed in vegetative characters (Figs. 1, 2) it is possibly due to phenotypic plasticity brought about by the harsher environmental conditions. Both species exhibited large variation within regions among individuals (Fig. 3a, b). The analysis of the first two principal components showed that, with the exception of Highland Switzerland, within-region variation between species exceeded the between region differentiation within species (Table 4a).

Moreover, univariate analyses showed that within-species regional differentiation differed between vegetative and reproductive characters (Table 3c, Figs. 2, 3). In Highland Switzerland *C. viridula* grew taller and had longer and wider leaves, resembling *C. flava* in that region (Fig. 1, Online Resource 1). Populations of *C. viridula* in Estonia and Lowland Switzerland differed mostly in the dimensions of their male spikes (SSL, SSPL, SSD; Fig. 2, Online Resource 1). Even though there is morphological variation within a species between regions it is not significant enough to merit the recognition of additional taxa. Accordingly, both univariate and multivariate analysis support the differentiation of *C. flava* and *C. viridula*.

Morphological differentiation between parental taxa and their hybrid *C.* x *subviridula*

Hybrids from Lowland Switzerland were intermediate between the parental species for one of 17 characters (7%), similar to parent *C. flava* for 9 characters (64%) and transgressive (more extreme than in *C. flava)* for 4 characters (29%; Table 5, Figs. 2, 3). Accordingly, hybrids from Lowland Switzerland differed significantly from *C. viridula* in 11 characters, but did not differ from *C. flava* in any of the measured characters. It is possible that those hybrids were more similar to *C. flava* due to backcrossing with it because *C. flava* is generally the more abundant sedge at the sites. However, it is unlikely that such backcrossing was repeated over several generations because fertility would then at least patially have been restored (Schmid 1980, 1982).

Hybrids from Estonia were intermediate between the parental species for 10 of 17 characters (71%), similar to the parent *C. viridula* in one character (7%) and transgressive (one character more extreme than in *C. flava* and two more extreme than *C. viridula)* for three characters (21%; Table 5, Figs. 2, 3). Accordingly, hybrids from Estonia differed significantly from *C. flava* in five reproductive characters and from *C. viridula* in four reproductive and three vegetative characters (Table 5).

The first two principal components explained 50% and 12% of the variation among the 14 morphological characters for all individuals of hybrids and parental species in Estonia and Lowland Switzerland combined (Fig. 3b). Thus, both univariate analyses (Table 5) and the PCA ordination clearly illustrated the similarity of the hybrids from lowland Switzerland to *C. flava* and the intermediacy of the hybrids from Estonia between the two parents (Fig. 3b). Accordingly, the taxa (i.e. *C. flava, C. viridula* and their hybrid) by region (Estonia and Lowland Switzerland) interaction was significant for PC1 (Table 4b).

Discussion

Morphological differentiation between *C. flava* and *C. viridula* in three regions

Based on univariate ANOVAs and multivariate PCA of 14 morphological characters, we found that the differentiation between *C. flava* and *C. viridula* in Estonia and Lowland Switzerland, but not in Highland Switzerland, exceeds the observed within-species differentiation between regions. This indicates that *C. flava* and *C. viridula* are distinct species in the southern and northern areas of their European distribution, despite their hybridization and introgression. It may suggest that the phenotypic divergence between the two species is maintained by selection against locally maladapted hybrid individuals, as has been observed e.g. for hybridizing *Senecio* species along a long elevational gradient on Mount Etna (Brennan

et al. 2016). Similarity of *Carex* species at the high altitudes in Switzerland could be due to local adaptation of *C. flava* or phenotypic plasticity in response to the harsher environment at higher altitude (Körner 1999). Alternatively, dwarfing of *C. flava* (shorter culm and leaf length) and increased leaf width and length of *C. viridula* in Highland Switzerland appears to account for the lack of differentiation rather than simply dwarfing of *C. flava* alone. Molecular markers should be used to test whether similarity of *Carex* species at the higher altitudes in Switzerland is due to genetic admixing (Schmidt et al. in prep.). Morphological differentiation between *C. flava* and *C. viridula* has been described within several regions (Schmid 1983; Crins and Ball 1989b, 1989a; Pykälä and Toivonen 1994; Hedrén 2002; Jiménez-Mejías et al. 2014), but we are the first to show that between-region differentiation is generally, though not always, smaller than between-species differentiation.

The taxa were more clearly delimited from each other in reproductive characters than in vegetative ones, as expected in general (Schmid 1992). This is also in line with previous research in *Carex*, where reproductive characters were considered to be more consistent and useful in delimiting closely related species (Davies 1953b; Crins and Ball 1989a), especially measures of utricles (Davies 1953a; Pykälä and Toivonen 1994; Salo et al. 1994; Hedrén 2002; Blackstock 2007; Janyszek et al. 2008). Thus, while the number of seeds and flowers may vary between different environments, their size is less variable (Janyszek et al. 2008).

Regional differentiation of *C. viridula* and *C. flava*

We found stronger regional differentiation in the less common species *C. viridula* (Table 3b, c; Fig. 3a). This is in line with our expectation that the species with the smaller and more scattered populations should show stronger regional differentiation. Our finding of high regional morphological variability of *C. viridula* is also consistent with earlier studies where *C. viridula* was found to show the highest phenotypic variability between populations within the complex in ecologically important vegetative characters (Schmid 1984b, 1986b; Stoeva and Stepankova 1990; Baur and Schmid 1996; Więcław 2011; Więcław and Podlasinski 2013). According to Pykälä and Toivonen (1994) the variation pattern of *C. viridula* in Fennoscandia is more complicated as the presence of interspecific taxa indicates its high evolutionary activity compared to other parts of the distribution range. This could be explained by a central-marginal hypothesis, as Fennoscandia is located in the periphery of the distribution. In another study the marginal populations of *Bromus tectorum* L. and *B. sterilis* L. showd differentiation into several lineages (Oja 1999). We encountered in Estonia also *C. viridula* varieties var. *bergrothii* (Palmgr.) B. Schmid and var. *pulchella* (Lönnr.) B. Schmid. But here we used only *C. viridula* var. *viridula*, so the observed differentiation supports the view of its evolutionary activity.

Experimental transplantations between the regions could reveal how much of the observed variation is due to heritable genetic differences among populations and how much due to environmental differences. Our results showed clear correlation between eco-geographic region and morphological characters, thus argued in favor of local selection.

Higher gene flow is expected between *C. flava* with its larger and less scattered populations than between the ones of *C. viridula* (Schmid 1984b, 1986a). Accordingly, we found little morphological variation between populations of *C. flava* from Lowland Switzerland and Estonia.

Carex flava from Highland Switzerland was morphologically differentiated from the populations of the same species from the two other studied regions, mostly by its smaller size of vegetative characters. Similarly, large variation in plant size of *C. flava* found in the Scandinavian material (Hedrén 2002) and in a comparative study between North America and northern parts of Eurasia was attributed to phenotypic plasticity (Crins and Ball 1989b). According to Schmid (1983) *C. flava* is replaced by *C. flava* var. *alpina* at high altitudes (over 1000 m) in central Europe. The var. *alpina* is identified by shorter stems (less than 30 cm) and leaves (less than 10 cm), which is similar to the size of *C. flava* from Highland Switzerland in our study (Online Resource 1). Therefore our results are in line with the recognition of the specimens of *C. flava* in Highland Switzerland as different ecotypes. We suggest to do reciprocal transplants between elevations to test whether the similarity of *C. flava* and *C. viridula* in Highland Switzerland is due to genetic differentiation or due to phenotypic plasticity.

Regional differentiation of the hybrid *C.* x *subviridula*

Hybrids are expected and often found to be intermediate between parents (Lamont et al. 2003; Lowe and Abbott 2003). We found hybrid intermediacy in Estonia, but not in Lowland Switzerland. In Estonian hybrids, 71.4% of the characters assayed were intermediate between parents, which is in accordance with previous reports for *C.* x *subviridula* (Stoeva and Stepankova 1990; Więcław 2014). In Lowland Switzerland the hybrids were mosaics with only 7.2% of the assayed characters intermediate, 64.2% *C. flava*-like and 29.6% extreme (Table 5, Fig. 3b). We suppose that the *C. flava*-like hybrid populations in Switzerland contained "cryptic backcrosses" *C. flava* x (*C.* x *subviridula*). This finding corresponds with that of Schmid (1982), who observed backcrosses at some field sites and suggested that *C. flava* can occasionally be pollinated successfully by F1- hybrids. Further backcrossing would then lead to increasingly more fertile and *C. flava*-like swarms of introgressive forms (Schmid 1982). Asymmetric introgression has been observed in various plant species and usually occurs towards the more abundat parent (e.g. Ducarme and Wesselingh 2005; Lepais et al. 2009). Our preliminary results

based on molecular makers show high admixture of the genotypes of morphologically identifyed hybrid individuals in Lowland Switzerland (Schmidt et al. in prep.). As we only studied few hybrid sites we are cautious with generalising to other hybridizing plants. It will be interesting to see for further species aggregates whether phenotypic hybrid–parent relations turn out to be very different in different regions.

Hybrids are mostly sterile and form relatively small populations (Wagner 1969; Schmid 1982) thus their longer-term persistence is questionable. However, transgressive segregation in hybrids is frequent and formed transgressive phenotypes could contribute to their persistence in nature (Rieseberg et al. 1999; Agbo and da Silva 2014). In Estonian hybrids we found transgressive characters for male spike dimensions (SSPL, SC, SSD). This would preadapt plants against selfing, as it increases the distance between female and male flowers. This phenomenon illustrates how hybridization may potentially contribute to ecological divergence via the generation of extreme characters in segregating hybrids, as proposed by Anderson (1949) and Rosenthal et.al (2002). The relatively high frequency of extreme characters in hybrids in our study supports the view of hybridzation as a source of variability upon which selection can act (Anderson 1949; Rieseberg et al. 1993; Rieseberg et al. 1999). It contradicts the arguments of Wagner (1969) that hybrids rarely contribute anything new to evolution and simply represent a blend of the parental species characters. It will be interesting to test the adaptive value of novel traits experimentally in the future (Rosenthal et al. 2002).

Conclusions

Based on our morphological data we conclude that hybridization and introgression occur between *C. flava* and *C. viridula*, but that genetic admixing is not strong enough to substantially increase the similarity between the two species within the different study regions. Hence, the parental species remain distinct in the southern and northern areas of their European distribution and between-region differentiation is clearly smaller than between-species differentiation. Thus, our study supports the idea that *C. flava* and *C. viridula* are separate species.

While regional differentiation within species occurred, region-specific genetic admixing between the two species seemed to have little effect. Thus, regional differentiation was more pronounced in the less common *C. viridula* than in the more widely distributed *C. flava,* which could be due to reduced gene flow between the more isolated and smaller *C. viridula* populations and increased genetic drift in these populations. The observed regional differentiation calls for using multiple environments and large geographic areas when studying closely related hybridizing taxa.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Information on Electronic Supplementary Material

Online Resource 1. Primary statistics (Mean and SE) of 14 morphological characters of *C. flava* and *C. viridula* in three regions.

References

Agbo CU, da Silva JAT (2014) Expression of Heterosis and Heritability in Vegetative Traits of *Gongronema latifolia*. O J Gen 4(2):146–156. doi: 10.4236/ojgen.2014.42015

Anderson E (1949) Introgressive hybridization, 1st edn. John Wiley & Sons, New York

Arnold ML (1992) Natural hybridization as an evolutionary process. Annual Rev Ecol Syst 23:237–261. doi: 10.1146/annurev.es.23.110192.001321

Arnold ML (1994) Natural hybridization and Louisiana irises. BioScience 44(3):141–147. doi: 10.2307/1312250

Arnold ML (2006) Evolution through genetic exchange, 1st edn. Oxford University Press Inc., New York

Baur B, Schmid B (1996) Spatial and temporal patterns of genetic diversity within species. *Biodiversity: a biology of numbers and difference.* Blackwell, Oxford

Blackstock N (2007) A reassessment of the yellow sedges-*Carex flava* L. agg. (Cyperaceae) in the British Isles. PhD Thesis, University of Lancaster

Blackstock N, Ashton PA (2010) Genetic markers and morphometric analysis reveal past hybridization and introgression in putative *Carex flava* L. s. str. (Cyperaceae) hybrid populations. Pl Syst Evol 287(1-2):37–47. doi: 10.1007/s00606-010-0287-0

Brennan AC, Hiscock SJ, Abbott RJ (2016) Genomic architecture of phenotypic divergence between two hybridizing plant species along an elevational gradient. AoB plants 8:plw022. doi: 10.1093/aobpla/plw022

Cayouette J, Catling PM (1992) Hybridization in the genus *Carex* with special reference to North America. Bot Rev 58(4):351–440. doi: 10.1007/BF02858773

Clausen J, Keck DD, Hiesey WM (1948) Experimental studies on the nature of species. III. Environresponses of climatic races of *Achillea*. Carnegie Institution., Washington

Crins WJ, Ball PW (1989a) Taxonomy of the *Carex flava* complex (Cyperaceae) in North America and northern Eurasia. I. Numerical taxonomy and character analysis. Canad J Bot 67(4):1032–1047. doi: 10.1139/b89-137

Crins WJ, Ball PW (1989b) Taxonomy of the *Carex flava* complex (Cyperaceae) in North America and northern Eurasia. II. Taxonomic treatment. Canad J Bot 67(4):1048–1065. doi: 10.1139/b89-138

Davies EW (1953a) An experimental taxonomic study of some species of *Carex* with special reference to the *C. flava* aggregate. PhD Thesis, University of London

Davies EW (1953b) Notes on *Carex flava* and its allies. III. The taxonomy and morphology of the British representatives. Watsonia 3:74–79

Davies EW (1953c) Notes on *Carex flava* and its allies. IV. Geographic distribution. Watsonia 3:80–84

Davies EW (1955) The cytogenetics of *Carex flava* and its allies. Watsonia 3:129–137

Ducarme V, Wesselingh RA (2005) Detecting hybridization in mixed populations of *Rhinanthus minor* and *Rhinanthus angustifolius*. Folia Geobot 40(2):151–161. doi: 10.1007/BF02803231

Egorova TV (1999) The sedges (*Carex* L.) of Russia and adjacent states (within the limits of the former USSR). St.-Petersburg State Chemical Pharmaceutical Academy, St.-Petersburg & Missouri Botanical Garden Press, St. Louis

Frankham R, Briscoe DA, Ballou JD (2002) Introduction to conservation genetics. Cambridge University Press, Cambridge, England

Galbany-Casals M, Carnicero-Campmany P, Blanco-Moreno JM, Smissen RD (2012) Morphological and genetic evidence of contemporary intersectional hybridisation in Mediterranean *Helichrysum* (Asteraceae, Gnaphalieae). Pl Biol 14(5):789–800. doi: 10.1111/j.1438-8677.2012.00568.x

Hahn MA, Rieseberg LH (2016) Genetic admixture and heterosis may enhance the invasiveness of common ragweed. Evol Appl 9:1–10. doi: 10.1111/eva.12445

Hall D, Luquez V, Garcia VM, St Onge KR, Jansson S, Ingvarsson PK (2007) Adaptive population differentiation in phenology across a latitudinal gradient in European aspen (*Populus tremula*, L.): a comparison of neutral markers, candidate genes and phenotypic traits. Evolution 61(12):2849–2860. doi: 10.1111/j.1558-5646.2007.00230.x

Harper JL (1977) Population biology of plants. Academic Press, Cambridge, Massachusetts

Hedrén M (1990) Problems in *Carex jemtlandica* and *C. bergrothii* (Cyperaceae) in Sweden. Sommerfeltia 11:109–115

Hedrén M (2002) Patterns of allozyme and morphological differentiation in the *Carex flava* complex (Cyperaceae) in Fennoscandia. Nordic J Bot 22(3):257–301. doi: 10.1111/j.1756-1051.2002.tb01373.x

Hegarty MJ, Hiscock SJ (2005) Hybrid speciation in plants: new insights from molecular studies. New Phytol 165(2):411–423. doi: 10.1111/j.1469-8137.2004.01253.x

Hultén E, Fries M (1986) Atlas of North European vascular plants (North of the Tropic of Cancer). Koeltz scientific books, Königstein

Janyszek M, Am Jagodziński, Janyszek S, Wrońska-Pilarek D (2008) Morphological variability of *Carex spicata* Huds. utricles among plant communities. Flora 203(5):386–395. doi: 10.1016/j.flora.2007.06.007

Jiménez-Mejías P, Luceño M, Martín-Bravo S (2014) Species boundaries within the southwest Old World populations of the *Carex flava* group (Cyperaceae). Syst Bot 39(1):117–131. doi: 10.1600/036364414X677973

Jiménez-Mejías P, Martín-Bravo S, Luceño M (2012) Systematics and taxonomy of *Carex* sect. *Ceratocystis* (Cyperaceae) in Europe: a molecular and cytogenetic approach. Syst Bot 37(2):382–398. doi: 10.1600/036364412X635449

Jonas CS, Geber MA (1999) Variation among populations of *Clarkia unguiculata* (Onagraceae) along altitudinal and latitudinal gradients. Amer J Bot 86(3):333–343. doi: 10.2307/2656755

Koopman J (2011) *Carex Europaea*: The Genus *Carex* L. (Cyperaceae) in Europe, 1: Accepted Names, Hybrids, Synonyms, Distribution, Chromosome Numbers. Margraf Publishers, Weikersheim

Körner C (1999) Alpine plant life: functional plant ecology of high mountain ecosystems. Springer-Verlag, Berlin

Kuchel SD, Bruederle LP (2000) Allozyme data support a Eurasian origin for *Carex viridula* subsp. *viridula* var. *viridula* (Cyperaceae). Madroño 47(3):147–158

Kukk T, Kull T (2005) Eesti taimede levikuatlas (Atlas of the Estonian flora). Estonian University of Life Sciences, Tartu

Lamont BB, He T, Enright NJ, Krauss SL, Miller BP (2003) Anthropogenic disturbance promotes hybridization between *Banksia* species by altering their biology. J Evol Biol 16(4):551–557. doi: 10.1046/j.1420-9101.2003.00548.x

Lepais O, Petit RJ, Guichoux E, Lavabre JE, Alberto F, Kremer A, Gerber S (2009) Species relative abundance and direction of introgression in oaks. Mol Ecol 18(10):2228–2242. doi: 10.1111/j.1365-294X.2009.04137.x

Lexer C, Joseph JA, van Loo M, Barbará T, Heinze B, Bartha D, Castiglione S, Fay MF, Buerkle CA (2010) Genomic admixture analysis in European *Populus* spp. reveals unexpected patterns of reproductive isolation and mating. Genetics 186(2):699–712. doi: 10.1534/genetics.110.118828

Lowe AJ, Abbott RJ (2003) A new British species, *Senecio eboracensis* (Asteraceae), another hybrid derivative of *S. vulgaris* L. and *S. squalidus* L. Watsonia 24(3):375–388

Oja T (1999) Allozyme Diversity and Interspecific Differentiation o the Two Diploid Bromegrass Species, *Bromus tectorum* L. and *B. sterilis* L (Poaceae). Pl Biol 1(6):679–686. doi: 10.1111/j.1438-8677.1999.tb00280.x

Pykälä J, Toivonen H (1994) Taxonomy of the *Carex flava* complex (Cyperaceae) in Finland. Nordic J Bot 14(2):173–191. doi: 10.1111/j.1756-1051.1994.tb00583.x

R Core Team (2015) R: A Language and Environment for Statistical Computing

Rieseberg LH (1997) Hybrid origins of plant species. Annual Rev Ecol Syst 28:359–389. doi: 10.1146/annurev.ecolsys.28.1.359

Rieseberg LH, Archer MA, Wayne RK (1999) Transgressive segregation, adaptation and speciation. Heredity 83(4):363–372. doi: 10.1038/sj.hdy.6886170

Rieseberg LH, Ellstrand NC, Arnold M (1993) What can molecular and morphological markers tell us about plant hybridization? Crit Rev Pl Sci 12(3):213–241. doi: 10.1080/713608045

Rius M, Darling JA (2014) How important is intraspecific genetic admixture to the success of colonising populations? Trends Ecol Evol 29(4):233–242. doi: 10.1016/j.tree.2014.02.003

Rosenthal DM, Schwarzbach AE, Donovan LA, Raymond O, Rieseberg LH (2002) Phenotypic differentiation between three ancient hybrid taxa and their parental species. Int J Pl Sci 163(3):387–398. doi: 10.1086/339237

Salo V, Pykälä J, Toivonen H (1994) Achene epidermis in the *Carex flava* complex (Cyperaceae) studied by scanning electron microscopy. Ann Bot Fenn 331:45–52

Santamaría L, Figuerola J, Pilon JJ, Mjelde M, Green AJ, Boer T, King RA, Gornall RJ (2003) Plant performance across latitude: the role of plasticity and local adaptation in an aquatic plant. Ecology 84(9):2454–2461. doi: 10.1890/02-0431

Schmid BW (1980) *Carex flava* L. sl im Lichte der r-Selektion. PhD Thesis, University of Zurich, Zurich

Schmid BW (1981) Die Verbreitung der Artengruppe *Carex flava* L. s.l. in der Schweiz. Bot Helv 91:3–8

Schmid BW (1982) Karyology and hydridization in the *Carex flava* complex in Switzerland. Feddes Repert 93(1-2):23–59. doi: 10.1002/fedr.19820930103

Schmid BW (1983) Notes on the nomenclature and taxonomy of the *Carex flava* group in Europe. Watsonia 14(4):309–319

Schmid BW (1984a) Life histories in clonal plants of the *Carex flava* group. J Ecol:93–114. doi: 10.2307/2260008

Schmid BW (1984b) Niche width and variation within and between populations in colonizing species (*Carex flava* group). Oecologia 63(1):1–5. doi: 10.1007/BF00379777

Schmid BW (1986a) Colonizing plants with persistent seeds and persistent seedlings (*Carex flava* group). Bot Helv 96(1):19–26

Schmid BW (1986b) Patterns of variation and population structure in the *Carex flava* group. Symb Bot Upsal 27(2):113–126

Schmid BW (1992) Phenotypic variation in plants. Evol Trends Pl 6(1):45–60

Stebbins GL (1959) The role of hybridization in evolution. Proc Amer Philos Soc 103(2):231–251

Stoeva MP, Stepankova J (1990) Variation patterns within the *Carex flava* agg. in Bulgaria and Czechoslovakia. Preslia 62(1):1–23

Toom M, Liira J, Kull T (2016) Tarnad - the genus *Carex* in Estonia [In Estonian], 1st edn. University of Tartu, Tartu

Vonk DH (1979) Biosystematic studies of the *Carex flava* complex I. Flowering. Pl Biol 28(1):1–20. doi: 10.1111/j.1438-8677.1979.tb01153.x

Wagner WH (1969) The role and taxonomic treatment of hybrids. BioScience 19(9):785–795. doi: 10.2307/1294787

West-Eberhard MJ (2003) Developmental plasticity and evolution, 1st edn. Oxford University Press, New York

Więcław H (2011) Morphological variability of the *Carex oederi* sl inflorescence. Biodivers Res Conservation 21:13–18. doi: 10.2478/v10119-011-0003-0

Więcław H (2014) *Carex flava* agg. (section Ceratocystis, Cyperaceae) in Poland: taxonomy, morphological variation, and soil conditions. Biodivers Res Conservation 33(1):3–51. doi: 10.2478/biorc-2014-0001

Więcław H, Podlasinski M (2013) Morphological differences between natural populations of *Carex viridula* (Cyperaceae): effects of soil conditions. Ann Bot Fenn 50(1-2):13–22. doi: 10.5735/085.050.0102

List of Figures

Table 1a Sampling sites of *Carex flava* and *C. viridula* populations in Highland Switzerland (CHH). Location gives latitude "N" and longitude "E", n = sample size, Elevation (m.a.s.l), $n_{(pop)}$ = total number of populations and $n_{(ind)}$ = total number of individuals

Taxa	Highland Switzerland (CHH)	Location N	E	Habitat	Elevation	n
C. flava	Arosa, Peist (Graubünden)	46.801	9.6841	wet meadow, lake shore	1920	21
C. flava	Cadagno, Quinto (Ticino)	46.548	8.7071	wet meadow, lake shore	1920	3
C. viridula						4
C. flava	Melchsee-Frutt, Kerns (Obwalden)	46.770	8.2809	wet meadow	1910	23
C. flava	Fähnern, Rüte (Appenzell Innerrhoden)	47.322	9.4695	wet meadow	1158	14
C. flava	Fontanivas, Disentis (Graubünden)	46.699	8.8565	wet meadow	1046	6
C. viridula						7
C. flava	Chapfensee, Mels1 (St. Gallen)	47.044	9.3826	bog	1046	5
C. flava	Chapfensee, Mels2 (St. Gallen)	47.048	9.3770	wet meadow, lake shore	1030	15
C. flava	Chapfensee, Mels3 (St. Gallen)	47.046	9.3854	skirts of the forest	1030	14
C. flava	Etang de Gruère, Saignelegier (Jura)	47.238	7.0508	shore of a ditch	1007	9
C. viridula				wet meadow		5
C. flava	Gupfloch, Rehetobel (Appenzell Ausserrhoden)	47.435	9.4988	forest path	1015	11
C. flava	$n_{(pop)}$ = 10, $n_{(ind)}$ = 121					
C. viridula	$n_{(pop)}$ = 3, $n_{(ind)}$ = 16					

Table 1b Sampling sites of *Carex flava*, *C. viridula* and hybrid *C. x subviridula* populations in Lowland Switzerland (CHL). Location gives latitude "N" and longitude "E", n = sample size, Elevation (m.a.s.l), $n_{(pop)}$ = total number of populations and $n_{(ind)}$ = total number of individuals

Taxa	Lowland Switzerland (CHL)	Location N	E	Habitat	Elevation	n
C. flava	Robenhuserriet, Wetzikon (Zürich)	47.340	8.7811	wet meadow	535	15
C. viridula						21
Hybrid						2
C. flava	Hudelmoos, Amriswil (Thurgau)	47.524	9.2869	footpath and wet meadow	525	15
C. viridula						14
Hybrid						4
C. flava	Neuweiher, Kreuzlingen (Thurgau)	47.631	9.1743	bog	500	31
C. viridula						26
Hybrid						9
C. flava	Kaltbrunner Riet, Kaltbrunn (St. Gallen)	47.215	8.9894	shore of ditch	410	19
C. viridula	Luganersee, Caslano, (Ticino)	45.961	8.8872	lake shore	270	20
C. flava	$n_{(pop)} = 4$, $n_{(ind)} = 80$					
C. viridula	$n_{(pop)} = 4$, $n_{(ind)} = 81$					
Hybrid	$n_{(pop)} = 3$, $n_{(ind)} = 15$					

Table 1c Sampling sites of *Carex flava*, *C. viridula* and hybrid C. x *subviridula* populations in Estonia (EST). Location gives latitude "N" and longitude "E", n = sample size, Elevation (m.a.s.l), $n_{(pop)}$ = total number of populations and $n_{(ind)}$ = total number of individuals

Taxa	Estonia (EST)	Location		Habitat	Elevation	n
		N	E			
C. viridula	Tarvastu, Veisjärv (Viljandimaa)	58.106	25.764	lake shore	97	10
C. flava	Helme, Holdre (Valgamaa)	57.964	25.743	wet meadow	93	21
C. flava	Helme, Lagesoo (Valgamaa)	57.949	25.807	bog	87	6
C. viridula						4
C. flava	Pajusi, Endla (Jõgevamaa)	58.760	26.131	shore of ditch	86	20
C. flava	Padriku, Anija (Harjumaa)	59.296	25.373	wet meadow	64	9
C. flava	Risti raba, Risti (Läänemaa)	58.993	24.074	bog	45	16
C. viridula						11
Hybrid						4
C. flava	Leie, Kolga-Jaani (Viljandimaa)	58.414	26.040	shore of ditch	37	20
C. viridula						19
Hybrid						20
C. flava	Luunja, Kabina (Tartumaa)	58.344	26.825	lake shore	33	18
C. viridula						16
Hybrid						7
C. viridula	Leisi, Meiuste (Saaremaa)	58.586	22.568	shore of ditch	27	20
C. viridula	Võhma, Mustjala (Saaremaa)	58.521	22.333	shore of ditch	19	18
C. viridula	Paatsa, Mustjala (Saaremaa)	58.505	22.313	shore of ditch	6	10
C. viridula	Nautse, Muhu (Saaremaa)	58.577	23.166	shore of ditch	3	10
C. flava	$n_{(pop)} = 7$, $n_{(ind)} = 110$					
C. viridula	$n_{(pop)} = 9$, $n_{(ind)} = 118$					
Hybrid	$n_{(pop)} = 3$, $n_{(ind)} = 31$					

Table 2 List of morphological characters measured in *C. flava*, *C. viridula* and hybrid specimens

Character	Units	Abbrevation
Quantitative characters:		
Plant height	cm	PH
Leaves length	cm	LL
Leaves width	mm	LW
Bracts length	cm	BL
Staminate spike length	mm	SSL
Staminate spike peduncle length	mm	SSPL
Female spike length	mm	FSL
Female spike width	mm	FSW
Utricle length (the sum of utricle body length and beak length)	mm	UL
Utricle width	mm	UW
Utricle beak length	mm	UBL
Qualitative characters:		
Stem curvature (1 = stem curved, 2 = stem straight)	Relative scale	SC
Staminate spike deflection (1= straight, 2= deflected, 3= strongly defl.)	Relative scale	SSD
Utricle beak curvature (1= curved, 2= slightly curved, 3= not curved)	Relative scale	UBC

Table 3 Geographic differentiation between a) *C. flava* and *C. viridula* populations across the three regions Estonia, Lowland Switzerland and Highland Switzerland and between the three regions for b) *C. flava* populations and for c) *C. viridula* populations

a) *C. flava* vs. *C. viridula*					b) *C. flava*	c) *C.viridula*
Trait	Taxa	Region	Taxa* Region	v.c.	Region	Region
df	1	2	2		2	2
PH	41.2 **	3.04	4.64 *	41	4.53 *	2.89
LL	15.9 **	0.96	3.81 *	39	3.13	2.09
LW	65.0 **	3.48 *	2.03	20	1.47	3.95
BL	5.80 *	0.79	3.49 *	24	1.99	4.57 *
SSL	3.01	4.05 *	3.13	22	0.06	11.8 **
SSPL	3.46	1.11	3.07	20	0.29	10.8 **
FSL	69.2 **	0.39	0.95	25	0.88	0.69
FSW	104.8 **	0.70	4.27 *	33	2.30	4.73 *
UL	331.6 **	0.72	5.22 *	27	2.78	3.01
UW	78.88 **	2.09	4.29 *	21	4.09 *	2.37
UBL	237.6 **	2.62	5.81 *	30	4.74 *	3.63
SC	2.30	3.60 *	0.70	17	4.11 *	2.86
SSD	3.70	1.40	1.10	19	0.01	2.71
UBC	43.2 ***	0.90	13.6 **	51	8.14 **	14.55 ***

Note: table entries in columns with asterisks are F values from ANOVA and levels of statistical significance: *, $P < 0.5$; **, $P < 0.01$; ***, $P < 0.001$; the column headed "v.c." shows variance components among populations within taxa and regions in % of sum of among- and within population residual variation. For abbreviations of characters see Table 2. For effect sizes see Figs. 2, 3

Table 4 Effects of taxa and region on principal components PC 1 and PC 2. a) *C. flava* and *C. viridula* in all three regions, b) Hybrid *C. x subviridula* vs. parental taxa in two regions

	df	PC 1 F		PC 2 F	
a) *C. flava* vs. *C. viridula*					
Taxa	1	171.28	***	2.76	
Region	2	1.57		3.23	
Taxa*Region	2	8.42	**	4.72	*
b) Hybrids vs. Parental taxa					
Taxa	2	82.49	***	3.05	
Region	1	0.95		5.68	*
Taxa *Region	2	4.26	*	2.96	

Note: degrees of freedom (df) and F values from ANOVA testing fixed (Taxa, Region, Taxa*Region) and random factors (Population). Symbols indicate levels of statistical significance: *, $P < 0.5$; **, $P < 0.01$; ***, $P < 0.001$. For abbreviations of characters see Table 2. For effect sizes see Fig. 3

Table 5 Primary statistics (Mean and SE) of 14 morphological characters of hybrid *(C.* x *subviridula*) populations and difference from parental taxa (*C. flava* and *C. viridula*) in the two regions Lowland Switzerland (CHL) and Estonia (EST). The state of hybrid characters vs. parental characters is indicated as "+" or "-" transgressive, intermediate, *C. flava* (CF)-like or *C. viridula* (CV)-like

	Lowland Switzerland	Comparison hybrid vs. C.*flava*	C.*viridula*	Estonia	Comparison hybrid vs. C.*flava*	C.*viridula*
Trait	Mean (SE)	F (df = 1)	F (df = 1)	Mean (SE)	F (df = 1)	F (df = 1)
PH	51.3 (17) (+transgr.)	0.02	11.0*	32.0 (5.0) (interm.)	4.39	19.7***
LL	30.3 (8.7) (+transgr.)	0.08	3.05	22.1 (3.8) (interm.)	0.77	8.98*
LW	4.54 (0.8) (+transgr.)	0.01	4.27	3.53 (0.4) (interm.)	1.28	8.34*
BL	8.20 (1.4) (interm.)	0.33	1.09	7.10 (1.6) (interm.)	2.54	2.05
SSL	14.8 (3.1) (+transgr.)	0.67	15.2**	13.9 (2.6) (interm.)	2.72	1.81
SSPL	4.40 (1.8) (CF-like)	0.01	13.2**	6.02 (2.1) (+transgr.)	2.20	3.33
FSL	9.70 (0.9) (CF-like)	0.73	23.8***	8.70 (1.3) (interm.)	3.62	6.13*
FSW	8.10 (0.9) (CF-like)	2.84	21.2**	6.30 (0.6) (interm.)	16.1**	7.32*
UL	4.30 (0.4) (CF-like)	1.59	97.4***	3.20 (0.2) (interm.)	60.5***	9.87**
UW	1.60 (0.2) (CF-like)	0.16	72.8***	1.40 (0.1) (interm.)	6.48*	4.54
UBL	1.70 (0.2) (CF-like)	0.01	155.9***	1.20 (0.1) (interm.)	22.5***	14.8**
SC	2.00 (0.0) (CF-like)	0.46	5.48*	1.60 (0.3 (-transgr.)	2.38	0.07
SSD	1.70 (0.4) (CF-like)	0.95	24.0*	1.10 (0.2) (-transgr.)	3.91	2.13
UBC	1.70 (0.4) (CF-like)	0.02	53.9***	3.00 (0.0) (CV-like)	19.6**	1.66

Note: degrees of freedom (df) and F values from ANOVAs for each trait. Symbols indicate levels of statistical significance: *, $P < 0.5$; **, $P < 0.01$; ***, $P < 0.001$. For abbreviations of characters see Table 2

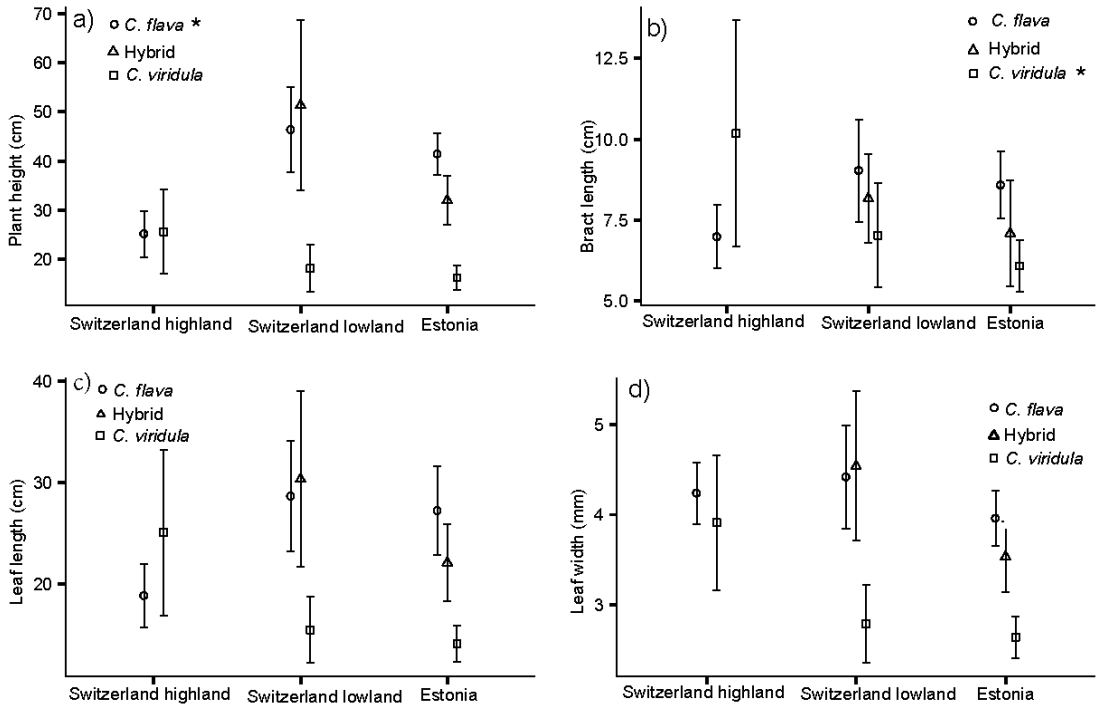

Fig. 1 Morphological variation in vegetative characters of *C. flava, C. viridula* and the hybrid in the three regions Highland Switzerland (CHH), Lowland Switzerland (CHL) and Estonia (EST). Plotted are mean values and standard errors, calculated among populations. Asterisks indicate within-species levels of between region significance: *, $P < 0.5$

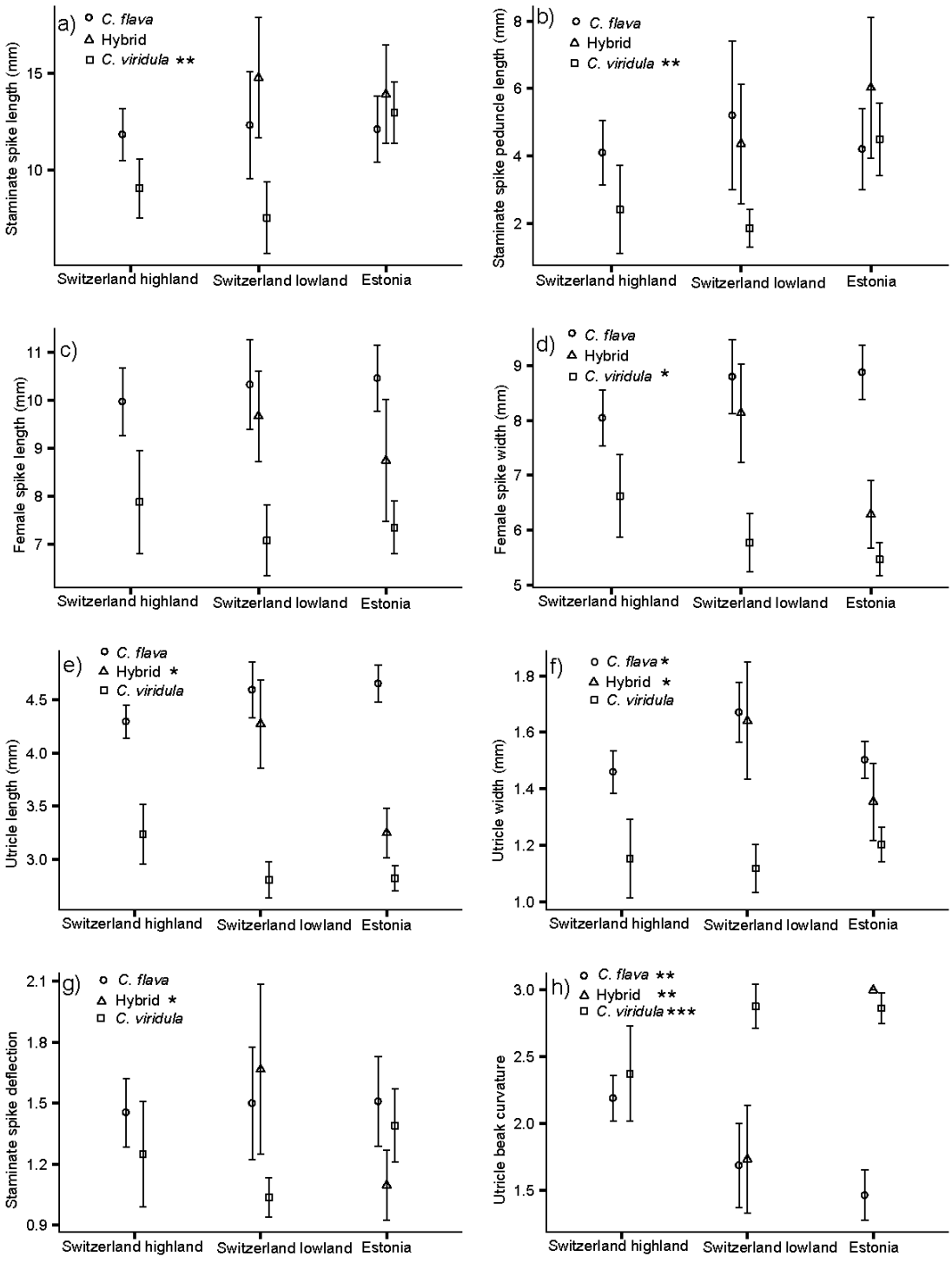

Fig. 2 Morphological variation in reproductive characters of *C. flava, C. viridula* and the hybrid in the three regions Highland Switzerland (CHH), Lowland Switzerland (CHL) and Estonia (EST). Plotted are mean values and standard errors, calculated among populations. Asterisks indicate within-species levels of between region significance: *, $P < 0.5$; **, $P < 0.01$; ***, $P < 0.001$

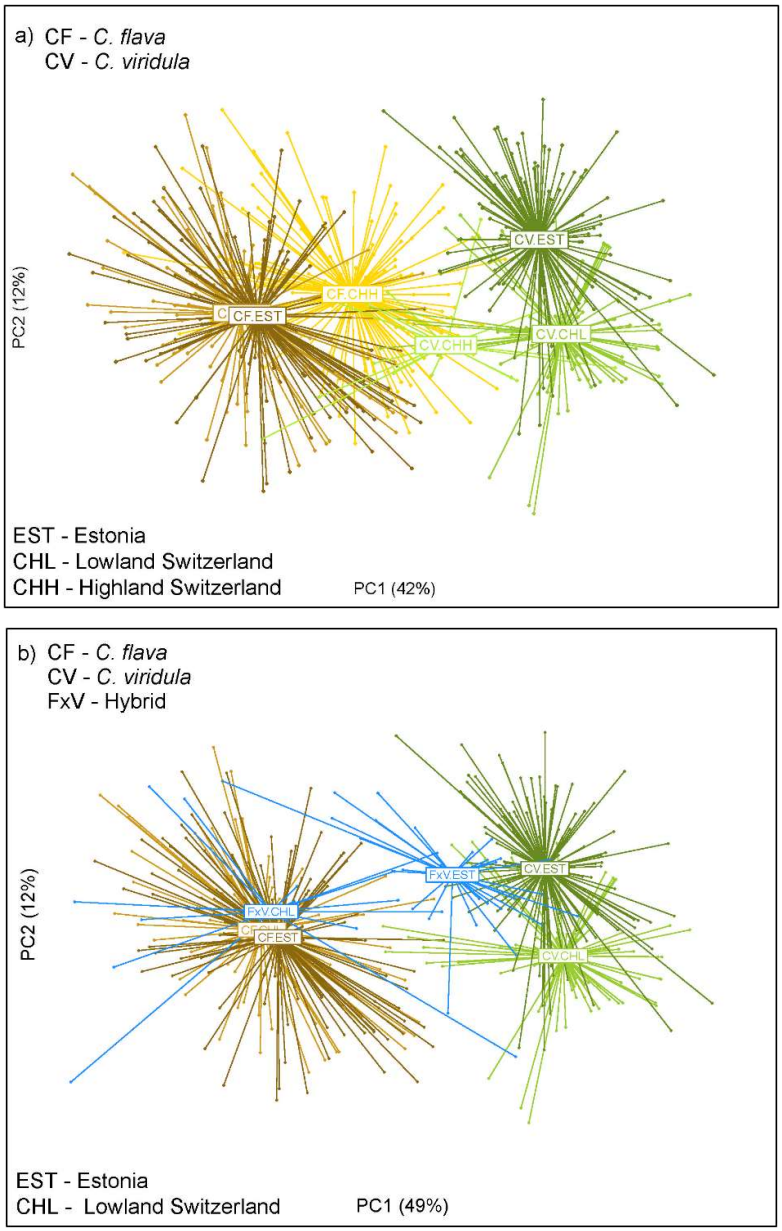

Fig. 3 Principal component analysis (PCA) of 14 morphological characters for a) *C. flava* and *C. viridula* individuals from Estonia (EST), Lowland Switzerland (CHL) and Highland Switzerland (CHH); b) for *C. flava, C. viridula* and their hybrid (*C.* x *subviridula*) from Estonia (EST) and Lowland Switzerland (CHL). The centroid of the phenotypic distribution of the taxa per origin is indicated by names in a rectangle to which each individual of that taxon and origin is connected by lines